MAGNESIUM SUPPLEMENTATION IN MARGINALLY DEFICIENT MICE

by

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A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

in

Nutrition

College of Health

The University of Utah

August 2016

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

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ABSTRACT

Magnesium (Mg) is an essential mineral that is important for bone health, energy production, and vasodilation; however, in the American diet, Mg intake is consistently below the Recommended Dietary Allowance (RDA). The aims of this project were to 1) assess which dietary form of Mg has the greatest bioavailability, 2) determine the effects of Mg depletion upon metabolic rate and energy expenditure, and 3) determine if Mg depletion alters vascular function. Sixty-four male C57BLK/6J mice were split into 4 groups for tissue concentration studies. One group consumed a diet containing a concentration of 100mg/kg Mg Oxide (n=22) for 3 weeks to produce Mg depletion. The other groups followed the same 3 week Mg depletion protocol (100mg/kg Mg Oxide), but then were given either 500mg/kg Mg Citrate (n=10), 500mg/kg Mg Malate (n=10), or 500mg/kg Mg Bisglycinate (n=22) for 1 week to replete Mg status. Mg Citrate and Mg Bisglycinate restored heart tissue Mg to equivalent concentrations while Mg Malate actually reduced heart Mg tissue concentration by 64% (p<.001). Mg Citrate restored bone Mg by 19% (p=.018), which was the greatest restoration. Liver Mg levels remained unchanged on all diets. Muscle Mg remained the same after replenishment with Mg Bisglycinate and was reduced by 62% on Mg Citrate (p<.001) and by 35% on Mg Malate (p<.001). A subset of 24 mice were used to address aims 2 and 3, and received 100mg/kg Mg Oxide diet for 3 weeks, or 100mg/kg Mg Oxide followed by repletion with 1 week on a 500mg/kg Mg Bisglycinate diet. Body fat was reduced by 35% in mice with 500mg/kg

Mg Bisglycinate (p=.005). Activity levels trended higher (p=.085), energy expenditure was higher (p=.001), and respiration rates were higher (p=.003) after repletion with 500mg/kg Mg Bisglycinate. Function of the vasculature was unchanged after repletion with 500mg/kg Mg Bisglycinate. In conclusion, these results indicate that Mg Citrate and Mg Bisglycinate are more effective at restoring tissue mg levels than Mg Malate. Further, low Mg intake may induce unfavorable body composition changes and reduce energy expenditure, but does not affect vascular function.

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INTRODUCTION

Magnesium (Mg) is an essential mineral that is important for bone health, energy production, and vasodilation (Bohl & Volpe, 2010). The typical American diet is high in refined foods and meat, which are low in Mg compared to diets rich in vegetables, whole grains, nuts, and seeds (Mahan & Escott-Stump, 2000), making it difficult to achieve recommended magnesium intakes. Multiple surveys, including the National Health and Nutrition Examination Survey III (NHANES III) (Cleveland, Goldman, & Borrud, 1994; Ford, 2003), and United States Department of Agriculture (USDA) Continuing Survey of Food Intake of Individuals II, have shown a median intake among the US population of 326mg per day for men and 234mg per day for women (Cleveland et al., 1994), which is below the Estimated Average Requirement (EAR) of 350mg per day for men and 265mg per day for women. Nutritional recommendations that encourage the adequate intake of whole grains and fruits and vegetables with an emphasis on nut and seed consumption may, in time, lead to more adequate dietary intake of Mg. Until Americans follow these guidelines, supplement use can be an important source of Mg intake (Ford, 2003).

Magnesium supplements come in different forms with varying bioavailability. Organic forms of Mg, such as citrate, aspartate, and fumarate, are better absorbed, utilized, and assimilated than the inorganic forms such as Mg oxide and hydroxide (Coudray, Rambeau, Feillet-Coudray, & Gueux, 2005; Fine, Ana, Porter, & Fordtran, 1991; Walker, Marakis, & Christie, 2003). The form of Mg that has shown the highest

rate of absorption and retention is a chelated form of Mg called Mg Bisglycinate (Schuette, Lashner, & Janghorbani, 1994; Siebrecht, 2013). Mg Bisglycinate is absorbed via the dipeptide transporter instead of competing with calcium for a mineral transport in the small intestine. Because it is chelated, it resists binding with phosphates, phytates, and tannins (Siebrecht, 2013). Specific details on how these supplemental forms of Mg replete various tissue concentrations following a period of low intake is needed to better assess the efficacy of each supplement.

Mg is needed for the metabolism of carbohydrate, lipid, and protein (Bohl & Volpe, 2010). Intracellular magnesium regulates insulin action and insulin-mediated glucose uptake, possibly by modulating phosphorylation and dephosphorylation events (Wolf & Trapani, 2008). Mg is needed for ATP production and utilization (Ko, Hong, & Pederson, 1999). Low Mg status may be related to decreased energy production, but there is a need to quantify the metabolic effects of low Mg status as it compares to a replete Mg status.

Decreased Mg status may be related to cardiovascular disease, hypertension, type II diabetes, osteoporosis, muscle wasting, muscle weakness, and muscle cramps (Bohl & Volpe, 2010; Classen, 1984; Ford, 2003; Rosanoff, Weaver, & Rude, 2012). Mg plays a role in the regulation of blood pressure through its competition with calcium for calcium binding sites on the smooth muscle cells of the vasculature. Mg deficiency may also influence the enzymes in the signal transduction pathway involved in vascular constriction (Sontia, 2007). Mg stimulates prostacyclin production and nitric oxide (NO) formation in the endothelial cells, which modulate vasodilation in the vasculature (Yang, Gebrewold, & Nowakowski, 2000). Mg also inhibits norepinephrine release from nerve endings, which causes a decrease in blood pressure (Shimosawa, Takano, & Ando, 2004). The antioxidant

and anti-inflammatory actions of Mg could attenuate damage to the vasculature from reactive oxygen species, and aid in preventing hypertension (Sontia, 2007). Though Mg may have an important role in vasodilation and constriction, it is unclear what level of Mg depletion may be required before vascular function is compromised.

The specific aims of this study were to 1) assess which dietary form of Mg has the greatest bioavailability by measuring liver, heart, muscle, and bone Mg concentration; 2) determine the effects of Mg depletion upon metabolic rate, energy expenditure, and concentration of metabolites in the liver; and 3) determine if Mg depletion alters vascular function.

We hypothesized that 1) Mg Bisglycinate would replenish tissues better than Mg Citrate and Mg Malate; 2) after consuming low Mg, restoration of Mg with Mg Bisglycinate would increase metabolic rate, energy expenditure; and 3) Mg depletion would impair vascular function compared to mice with restored Mg levels.

MATERIALS AND METHODS

Mice and groups with timeline

All protocols used in the study were approved by the Animal Use and Care Committee at the University of Utah and conformed to guidelines set by the American Physiological Society and Animal Welfare Act. Sixty-four C57BL/6J male mice were obtained from The Jackson Laboratory Inc. (Bar Harbor, ME). Mice started diets at 8 weeks old. Mouse chow was obtained from Research Diets Inc. (New Brunswick, NJ). Mice were housed 4 or 5 to a cage on 12 hour light / 12 hour dark cycles. Deionized water was used for drinking water. Cages were changed daily to keep mice from ingesting their feces to prevent Mg recycling. Food and water was ad libitum. Mice were divided into the following experimental groups for this study.

Group 1 – *N*=22 (will be referred to as low Mg group). Mice underwent 3 weeks of magnesium restriction using a diet of 100mg/kg Mg Oxide (Carney, Wong, & Quamme, 1980; Feillet-Coudray, Coudray, & Wolf, 2004). Mg tissue content, body compostion, metabolic function, liver metabolite levels, and vessel reactivity were measured.

Group 2 – *N*=22 (will be referred to as restored Mg group). Mice underwent 3 weeks of magnesium restriction using a diet of 100mg/kg Mg Oxide followed by 1 week of magnesium replenishment with a diet of 500mg/kg Mg Bisglycinate. Mg tissue content, body compostion, metabolic function, liver metabolite levels, and vessel

reactivity were measured.

Group 3 – *N*=10. Mice underwent 3 weeks of magnesium restriction using a diet of 100mg/kg Mg Oxide followed by one week of replenishment using a diet of 500mg/kg Mg Citrate. Mg tissue content was measured.

Group 4 – *N*=10. Mice underwent 3 weeks of magnesium restriction using a diet of 100mg/kg Mg Oxide followed by one week of replenishment using a diet of 500mg/kg Mg Malate. Mg tissue content was measured.

Euthanizing

Mice were anesthetized using 3-5% isoflurane until the requisite plane of anesthesia was obtained. Next, the chest was opened. A blood sample via a cardiac puncture was obtained (750 ul-1000ul) and urine was collected via bladder puncture. The heart was then excised, and tissues obtained.

Tissue analysis

Heart, liver, muscle (gastrocnemeus, soleus, and quadracep), and tibia bone were obtained after heart excision. Blood was centrifuged at 2500rpm for 15 minutes at 4°C and plasma was seperated by pipette. Tissues were dried and ashed at 500°C for 10 hours and the ash was dissolved in 0.2 mL of concentrated HNO3 and 9.2 mL of distilled water. For total Mg determination, samples were diluted appropriately with 0.1% lanthanum chloride and Mg level was determined by flame atomic absorption spectrometry (Perkin Elmer 560, Courteboeuf, France) (Coudray et al., 2005).

Nuclear magnetic resonance (NMR)

Fat mass, lean mass, and fluid mass were determined using NMR. Mice were individually weighed and placed inside chamber tube for measurements (Minispec Bruker Corp., Bellerica, MA).

Metabolic chamber

The Columbus Instruments (Columbus, OH) Comprehensive Laboratory Mouse Monitoring System (CLAMS) metabolic chamber is an open circuit system that directly measures five parameters. Mice were housed singly with water and respective diets available ad libitum, and maintained at 20–22 °C under a 12:12-h light–dark cycle (light period 0600–1800 hours). Sample air was passed through an oxygen sensor for determination of oxygen content. Oxygen consumption was determined by measuring oxygen concentration in air entering the chamber compared with air leaving the chamber. The sensor was calibrated against a standard gas mix containing defined quantities of oxygen, carbon dioxide, and nitrogen. Constant airflow (0.6 l/min $^{-1}$) was drawn through the chamber and monitored by a mass-sensitive flowmeter. The concentrations of oxygen and carbon dioxide were monitored at the inlet and outlet of the sealed chambers to calculate oxygen consumption. The system has beams 0.5 inches apart on the horizontal plane, providing a high-resolution grid covering the XY planes and the software provides counts of beam breaks by the mouse in 30-second intervals (Martin-Montalvo, Merken, & Mitchell, 2013). After allowing 18 hours for mice to acclimate to housing in the metabolic chamber, parameters of 1) oxygen consumption, 2) carbon dioxide production, 3) food intake, 4) water intake, 5) and activity over X Y & Z axis were measured over a

continuous 48-hour period (see Figure 1). The University of Utah Metabolic Phenotyping Core performed these analyses.

Glucose tolerance test (GTT)

Mice fasted for 6 hours in individual cages with water only. After body weights were recorded, glucose diluted in saline was injected at 1mg/g body weight intraperitoneally. Blood glucose levels were recorded from a nick at the tip of the tail at 0, 5, 15, 30, 60, and 120 minutes.

Metabolomics

Metabolomic analysis was performed at the Metabolomics Core Facility at the University of Utah. Metabolites were identified based on known retention times and mass fragmentation patterns. The peak area for each metabolite was recorded, which can be used to compare groups in terms of relative abundance of each metabolite. The metabolites that were measured from liver samples were lactic acid, pyruvic acid, glycolic acid, glycerol, glyceric acid, citric acid, aconitate, isocitric acid, 2-ketoglutaric acid, succinic acid, fumaric acid, malic acid, 2-hydroxyglutarate, 2-aminoadipic acid, lysine, valine, leucine, isoleucine, threonine, homoserine, glycine, serine, alanine, glutamic acid, glutamine, proline, aspartic acid, asparagine, methionine, cysteine, homocysteine, phenylalanine, tyrosine, tryptophan, histidine, sarcosine, 4-hydroxyproline, ornithine, N-acetylglutamate, N-acetylaspartate, B-alanine, phosphate, diphosphate, 1-phosphoglyceril, 2-phosphoglycerol, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, fructose, mannose, ribose, glucose, glucose-1-phosphate, fructose-6-phosphate, glucose-6-phosphate, sorbitol, gluconic acid, inositol,

myo-inositol phosphate, sedoheptulose-7-P, sucrose, erythrose, erythose-4-phosphate, ribitolor xylitol, ribose(or xylose)-5-phosphate, ribulose(or xylose)-5-P, fucose, myristic acid, palmitiladic acid, 17:0 fatty acid, palmitic acid, linoleic acid, oleic acid, elaidic acid, stearic acid, 19:0 fatty acid, arachidonic acid, 20:1 fatty acid, 22:0 fatty acid, 24:0 fatty acid, 24:1 fatty acid, 1-monolinoleoylglycerol, 1-monooleoylglycerol, 1-monostearylglycerol, 1-monopalmitoylglycerol, 2-monostearylglycerol, cholesterol, pantothenic acid, phosphoethanolamine, xanthine, hypoxanthine, adenosine, inosine, adenine, thymine, cytosine, uracil, adenosine-5'-monophosphate, inosine-5'-monophosphate, kynurenine, tocopherol, 4-aminobutyrate, urea, creatinine, 3-hydroxybutyrate, uric acid, serotonin, dopamine, 1,3-bisphosphoglycerate, 2-hydroxybutyric acid, sucrose, nicotinamide, GSH reduced, 5-aminopentanoic acid, 2,3-pyridinedicarboxylic acid, 5-hydroxytryptophan, porphobilinogen, and oleamide.

Vessel experiments

Immediately following euthanizing, femoral arteries were isolated distal to the bifurcation of the internal iliac artery. Two vessel segments per mouse were treated identically and the results were averaged. During dissection, tissues were bathed in ice-cold, oxygenated normal physiological saline solution (NPSS). Once isolated and free of adherent tissue, femoral arteries were mounted on a wire-type myograph while immersed in temperature-controlled, 8-ml tissue "bath" containing oxygenated (95% O_2 -5% CO_2) NPSS (pH 7.35 - 7.45). After the arteries were mounted, the tissue bath was gradually warmed to 37°C over 30 minutes with vessels at 0-mg tension. During this time and throughout each experiment, the pH and temperature of all buffer solutions were checked at 30-minute intervals, and contents of the tissue bath were exchanged at 15-minute

intervals.

When the tissue bath reached 37°C, tension on femoral arteries was increased manually over 60 minutes to 2g. After 30 minutes, a series of internal circumference-active tension curves was constructed to determine the vessel diameter that evoked the greatest tension development to 100mM KCL. Receptor-mediated vasocontractile responses to phenylephrine (PE 10^{-8} – 10^{-5} M) and potassium chloride (KCL, 10-100mM) were assessed 30 minutes later. Next, after arteries were precontracted to 65% of maximal PE induced contraction and tension was stable, responses to acetylcholine (Ach, 10^{-8} – 10^{-4} M; to determine endothelium dependent vasorelaxation), and sodium nitroprusside (SNP, 10^{-9} – 10^{-4} M; to determine endothelium independent vasorelaxation) were performed. Each protocol was separated by at least 30 minutes. All tension data were recorded continuously by a computer through an analog-to-digital interface card (Biopac Systems Inc., Santa Barbara, CA) that allowed for subsequent off-line quantitative analyses (Symons, McMillin, & Riehle, 2008).

Statistics

Statistics were done using SPSS version 20 with an accepted significance level of $p<.05$. Data are presented as a mean +/- standard error (SE). An unpaired *t*-test was used for NMR, GTT, and to compare each cycle (light and dark) between the low vs. restored groups in the metabolic chamber (CLAMS). For these analyses, significance is denoted in the figures by an asteriks (*). A one-way ANOVA was used to test Mg tissue concentration. For these analyses, significance is denoted in the figures by using letters such that different letters denote significant differences among groups. An ANOVA with repeated measures was used to test vessel reactivity data.

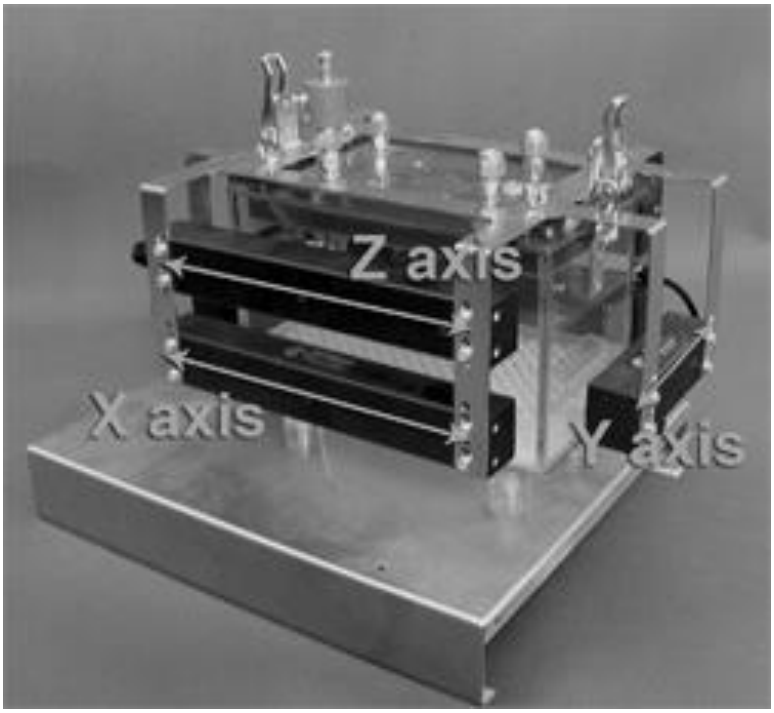


Figure 1 CLAMS metabolic cage with movement axis labeled.

RESULTS

Tissue analysis

Group 1-4 mice were tested for Mg concentration in liver, heart, muscle (gastroc, soleus, quadracep), and tibia bone tissues. No significant differences were found in liver tissue Mg concentration (see Figure 2a). Compared to mice fed low Mg diet (100 mg/kg chow Mg Oxide), mice fed a repenishment diet of 500 mg/kg Mg Citrate and 500 mg/kg Mg Bisglycinate had significantly greater Mg concentration in the heart than mice fed a replenishment diet of 500 mg/kg Mg Malate (see Figure 2b). In muscle tissue, there was no difference in Mg concentration between the 100 mg/kg Mg Oxide diet and the 500 mg/kg Mg Bisglycinate diet; however, both of these were greater than the 500 mg/kg Mg Malate and 500 mg/kg Mg Citrate diets (see Figure 2c). Compared to mice fed low Mg diet, bone Mg concentrations were greater after replenishment with 500 mg/kg Mg Citrate compared to all groups (see Figure 2d), and there was a trend toward lower Mg bone concentrations after replenishment with 500 mg/kg Mg Malate vs. 100 mg/kg Mg Oxide (p=.051) (see Figure 2d). Replenishment using 500 mg/kg Mg Bisglycinate partly increased bone Mg content such that concentrations were in between levels found in mice fed 100 mg/kg Mg Oxide diets and mice replenished with 500 mg/kg Mg Citrate, and statistically equivalent to both (see Figure 2d).

Final body weight and composition

Final body weight, fat mass, percent body fat, lean body mass, percent lean body mass, fluid mass, and percent fluid mass were determined. The restored Mg group had significantly less fat mass and percent body fat than the low Mg group. A significant difference was also found in the percent fluid mass measurement, with the restored Mg group having a higher percent fluid mass than the low Mg group ($p<.001$) (see Figures 3a-g).

Metabolic chamber

The restored Mg group had significantly more beam brakens in the Y plane during the inactive light cycle at the $p<.05$ level. There was a trend toward the restored Mg group having more beam brakes in the X plane during the active dark cycle, in the Y plane during the active dark cycle, and in total beam brakes in a 48-hour period ($p\leq.10$) (see Figure 4a-d). Taken together, these data indicate increased physical activity (movement) in mice while on the restored Mg diet.

Metabolic rate and gas exchange

Gas exchange of VO2 and VCO2 was measured in the metabolic cages. A significant increase in VO2 respired and VCO2 expired was seen in the restored Mg group relavite to the low Mg group ($p<.05$) (see Figure 5a,b).

Respiratory exchange ratio (RER), food and water intake,
energy expenditure

No significant differences were noted in RER, food, or water intake between groups during active cycle or rest cycle (see Table 1). Energy expenditure was also

measure in the metabolic cages in Kcal per hour. The low Mg group had less energy expenditure than the restored Mg group ($p<.05$) (see Figure 6).

Glucose tolerance test (GTT)

Tolerance to a glucose challenge was measured. Glucose levels at baseline and at each time point up to 2 hours after intraperitoneal glucose challenge were similar between low Mg and restored Mg groups (see Figure 7).

Metabolomics

In analysing mice liver tissue, significant differences were found in the levels of glycine, inosine, and reduced glutathione, with each of these metabolites being in higher quantities in the restored Mg group when compared to the low Mg group. The fatty acid 22:0 had a higher quantity in the low Mg group compared to the restored Mg group. None of the other metabolites measured had significant differences (see Table 2).

Vessel function testing

Femoral artery vessels showed no significant difference in start width, end width, vessel length, and maximum tension force (see Table 3).

Femoral artery vessels showed no significant difference in endothelium dependent or endothelial independent function between low Mg and restored Mg groups when challenged with acetylcholine, sodium nitroprusside, phenylephrine, and potassium chloride. Vessel response to acetylcholine, an endothelium dependent and receptor dependent arterial vasodilator, was similar among groups (see Figure 8a). Vessel dilation in response to sodium nitroprusside, an endothelium independent and receptor independent vasodilator, was similar (see Figure 8b). Contractile response to

phenylephrine, a receptor dependent vasoconstrictor, was not different among groups (see Figure 8c), as was contraction response to potassium chloride, a receptor independent vasocontrictor (see Figure 8d).

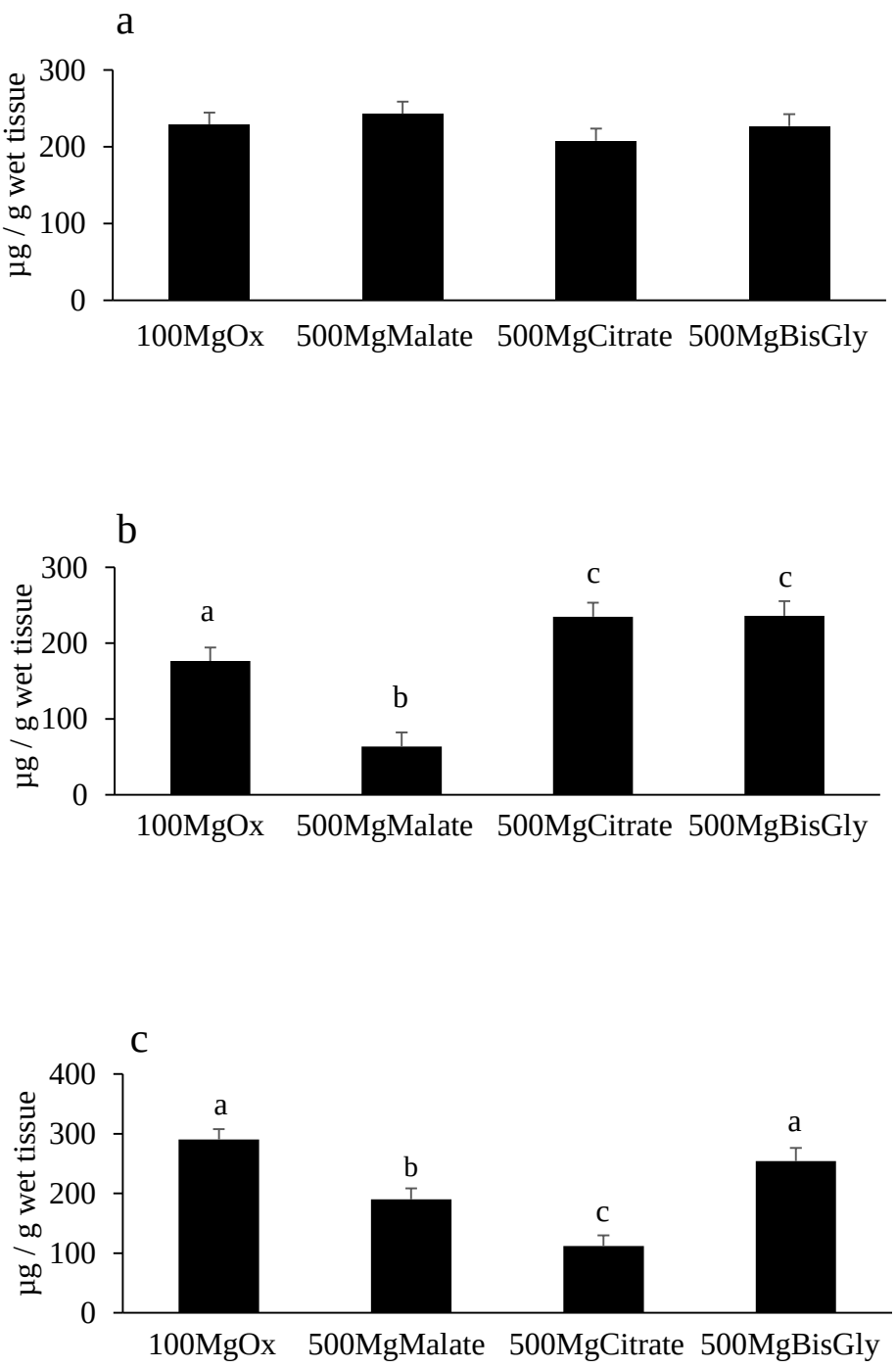
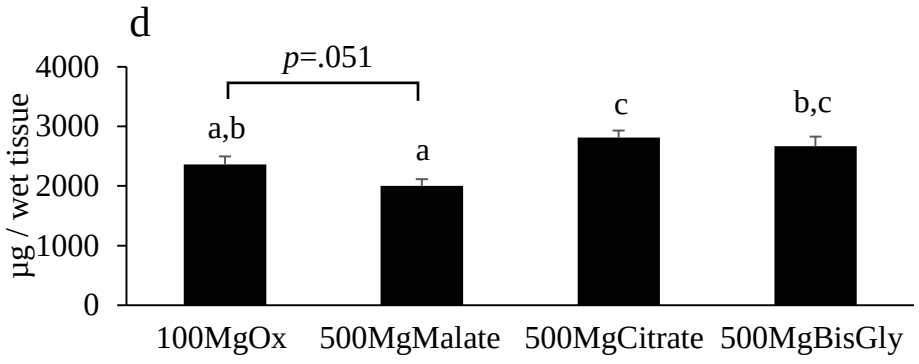


Figure 2 Liver, heart, muscle, and bone tissue concentrations of Mg. Different letters signify significant differences at $p<.05$. a) Liver. All groups had $n=10$. b) Heart. 100MgOx $n=11$, 500MgMalate and 500MgCitrate $n=10$, 500MgBisGly $n=9$. c) Muscle. 100MgOx $n=11$, 500MgMalate and 500MgCitrate $n=10$, 500MgBisGly $n=7$. d) Bone. 100MgOx $n=7$, 500MgMalate $n=10$, 500MgCitrate $n=9$, 500MgBisGly $n=5$.



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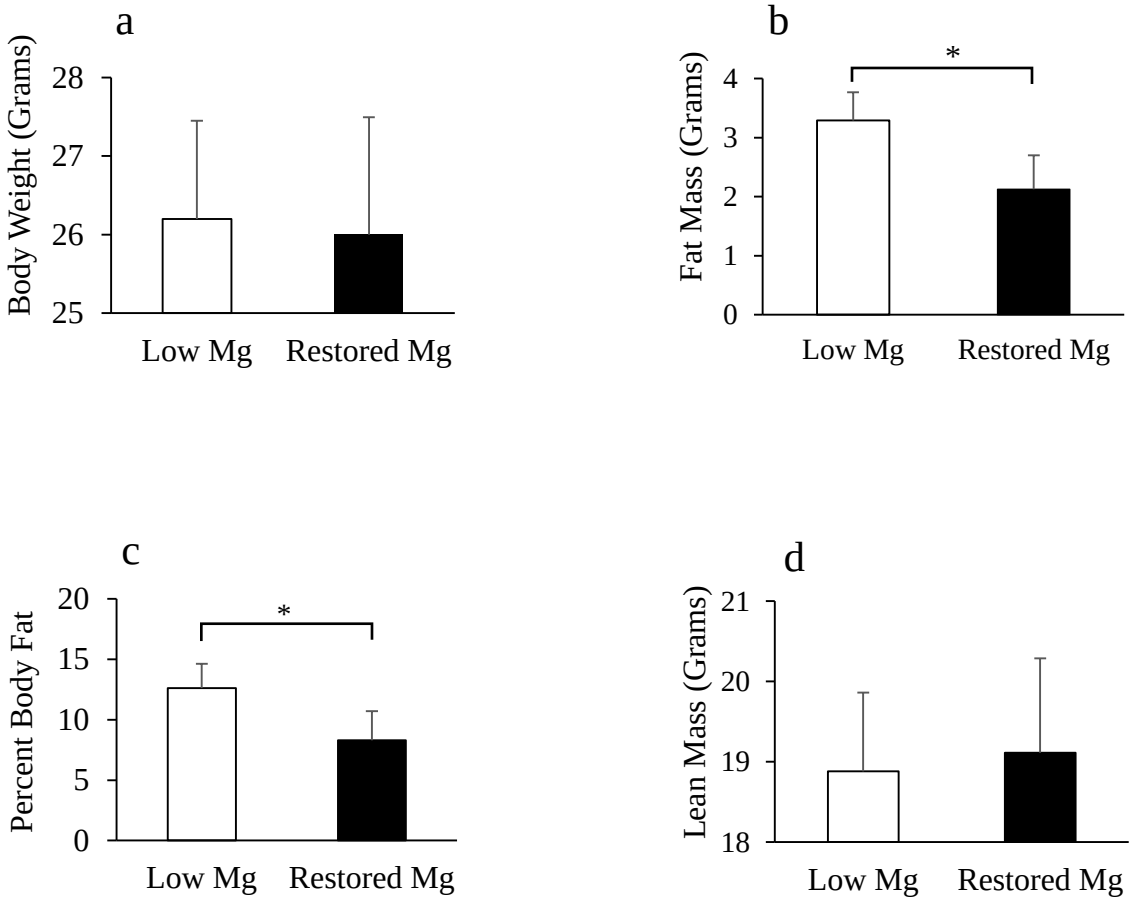
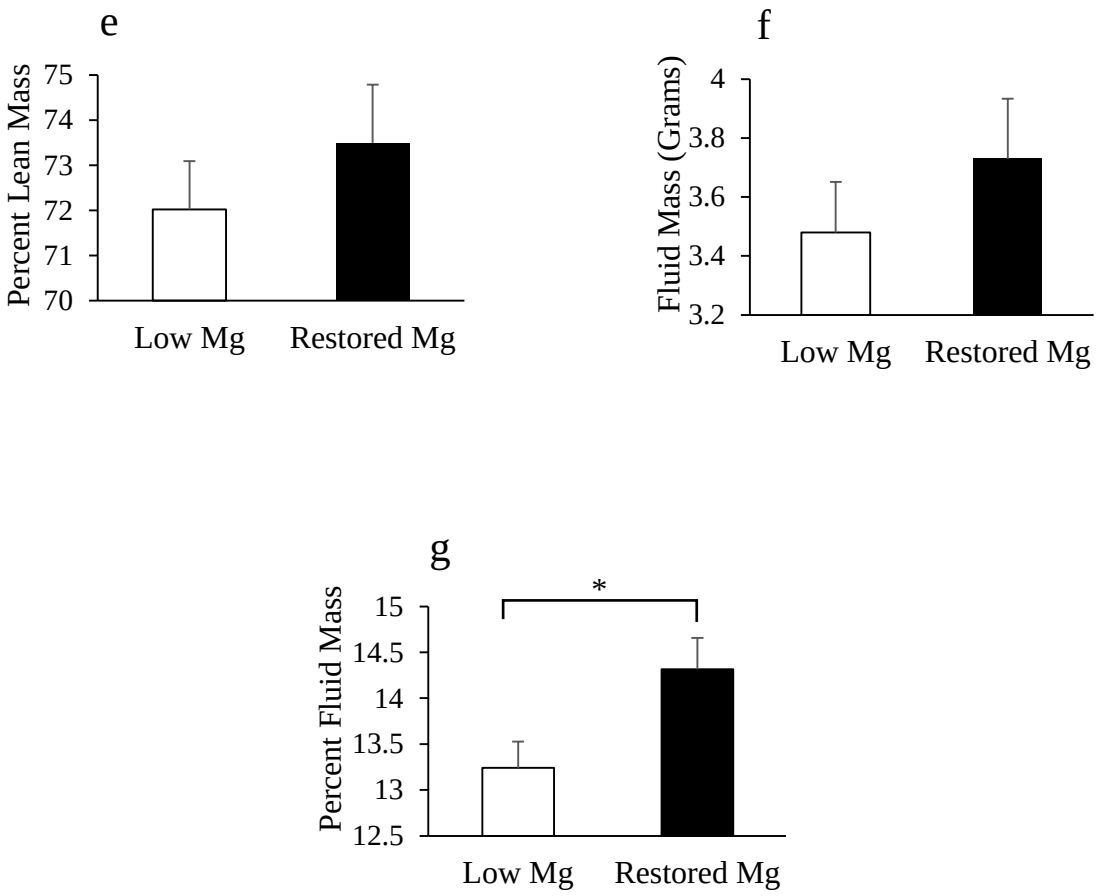


Figure 3 Results of body composition analysis. Asterisks * denote significant differences; $p<.05$. Low Mg group $n=10$, restored Mg group $n=7$. a) Final body weight. b) Fat mass. c) Percent body fat. d) Lean body mass. e) Percent lean body mass. f) Fluid mass. g) Percent fluid mass..



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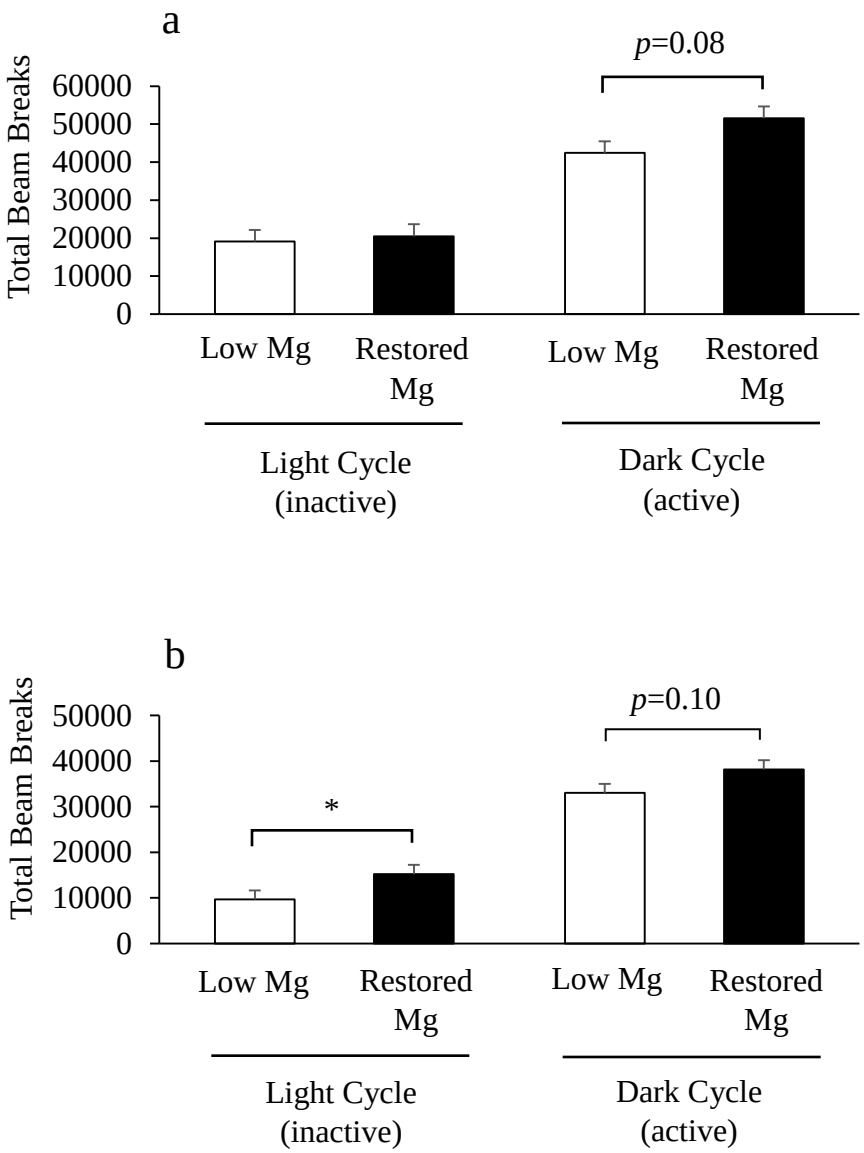
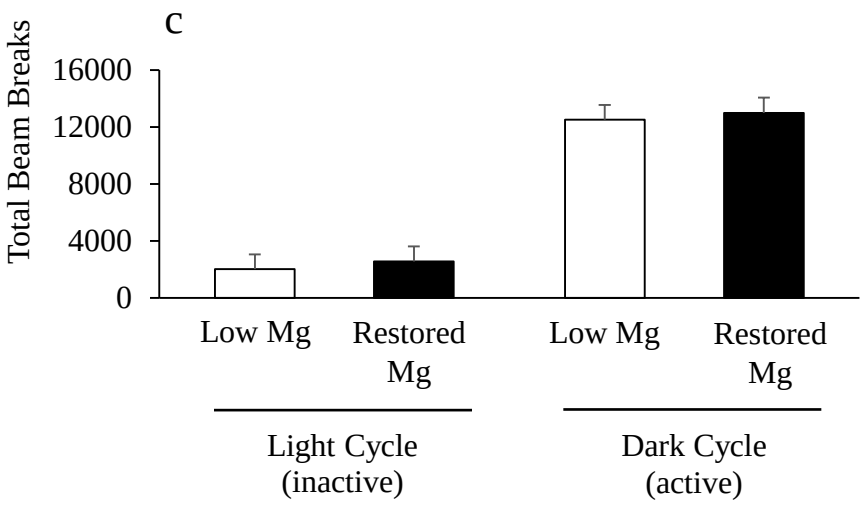
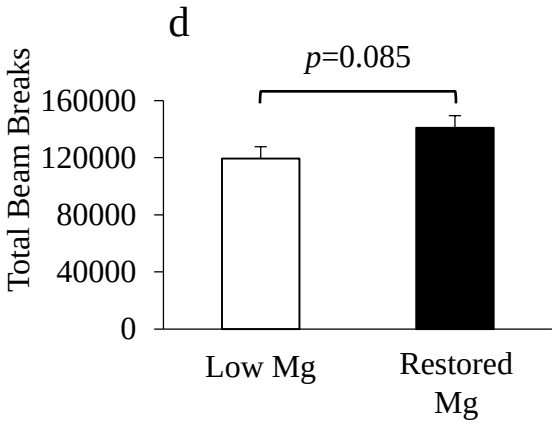


Figure 4 Physical activity parameters. Reported as beam breaks in the X, Y, and Z plane. Low Mg group *n*=11, restored Mg group *n*=12. Asterisk * denotes significant differences; *p*<.05. a) X plane movement. b) Y plane movement. c) Z plane movement. d) 48-hour X,Y,Z combined movement.





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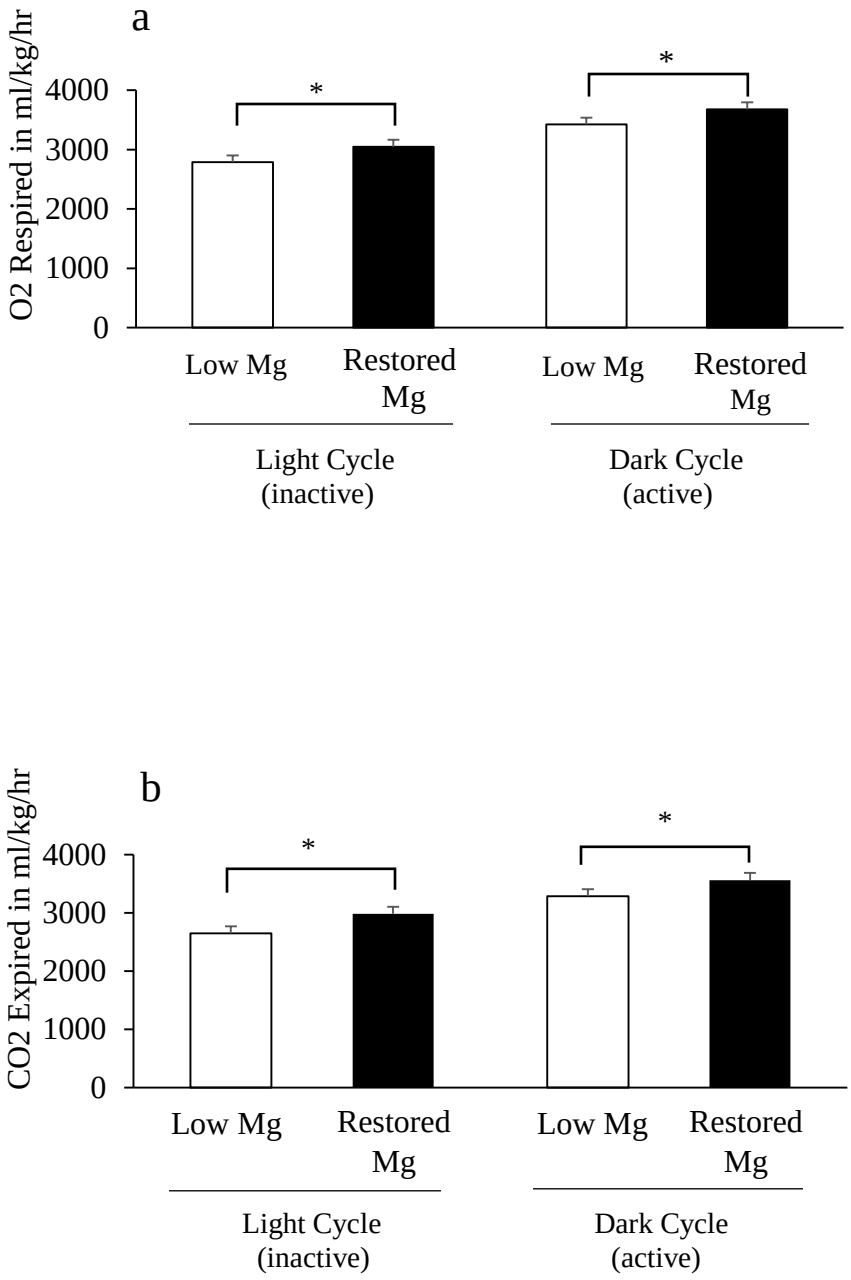

Figure 5 Gasses exchanged in metabolic cages. Low Mg group *n*=11, restored Mg group *n*=12. Asterisks * denote significant differences; *p*<.05. a) VO2 respired. b) VCO2 expired.

Table 1 RER, food, and water intake (combined over two cycles) in grams.
Low Mg group *n*=11, restored Mg group *n*=12

	Low Mg Active Cycle	Restored Mg Active Cycle	Low Mg Rest Cycle	Restored Mg Rest Cycle
RER	.961 +/- .013	.969 +/- .014	.952 +/- .013	.979 +/- .014
Food Intake	4.325 +/- .271	4.38 +/- .283	3.584 +/- .271	4.1575 +/- .283
Water Intake	3.74 +/- .087	3.34 +/- .091	2.203 +/- .087	2.25 +/- .091

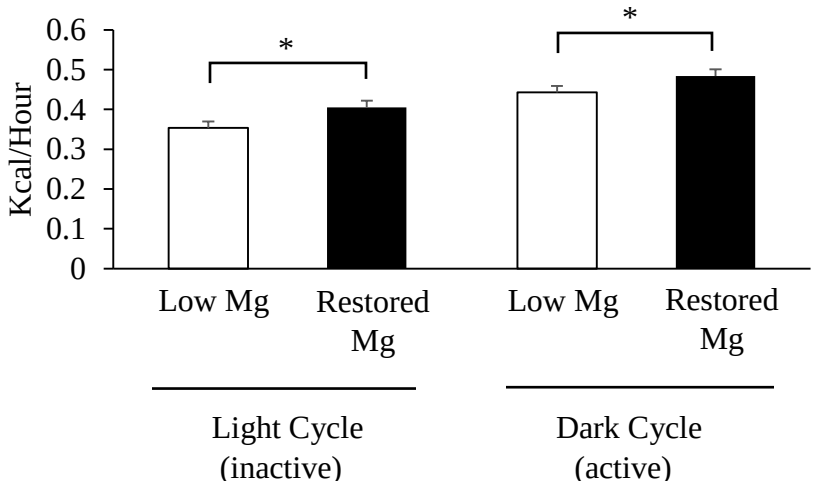

Figure 6 Energy expended reported in kcal/hour. Low Mg group *n*=11, restored Mg group *n*=12. Asterisks * denote significant differences; $p<.05$.

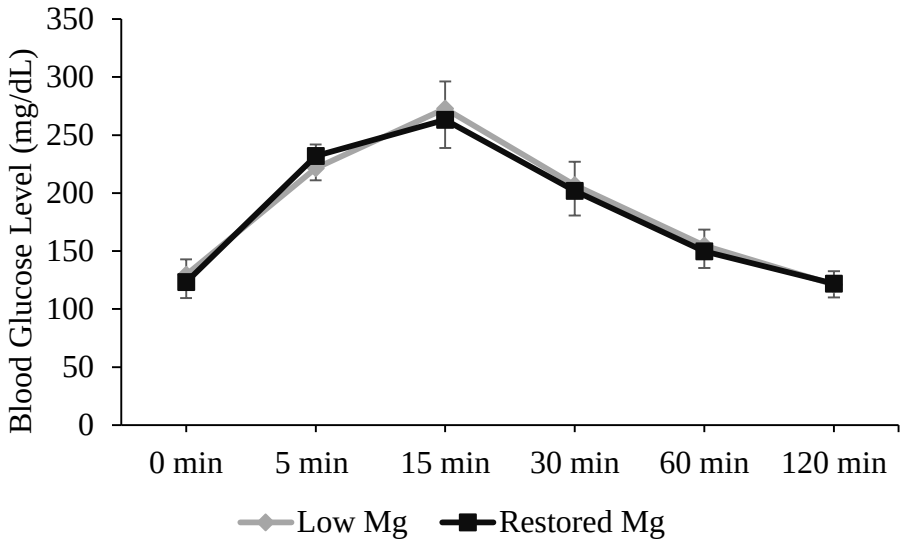

Figure 7 Glucose tolerance tests (GTT) results. Reported as measured blood glucose level in mg/dL. Low Mg group *n*=11, restored Mg group *n*=12. Asterisks * denote significant differences at $p<.05$.

Table 2 Hepatic analysis of metabolites with significant differences.
Data expressed as mean +/- SE in units of relative abundance.
Low Mg group *n*=7, restored Mg group *n*=7

Metabolite	Low Mg	Restored Mg	*P* value
Glycine	1401.3 +/- 68.9	1906.1 +/- 163.24	.015
22:0 Fatty acid	10.23 +/- 1.1	4.22 +/- 1.34	.005
Inosine	54.05 +/- 12.6	152.06 +/- 39.58	.036
Reduced Glutathione	2902.1 +/- 478.1	4996.18 +/- 697.66	.029

Table 3 Vessel characteristics. No significance between groups.
Values are presented as mean +/- SE.
Low Mg group *n*=9, restored Mg group *n*=10

	Start Width (µm)	End Width (µm)	Vessel Length (µm)	Max Tension Force (mg tension)
Low Mg	172.6 +/- 7.7	477.8 +/- 5.3	1832.2 +/- 29.7	2537.89 +/- 112.3
Restored Mg	167.0 +/- 5.8	471.3 +/- 8.6	1812.5 +/- 31.0	2520.15 +/- 132.2

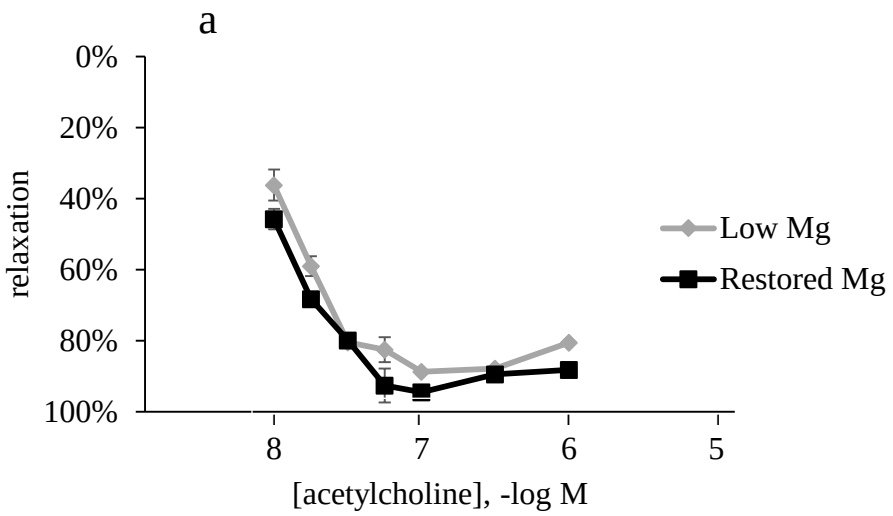

Figure 8 Results of vessel functions tests. Low Mg *n*=9, Restored Mg *n*=10 a) Endothelial and receptor dependent arterial relaxation. b) Endothelial and receptor independent arterial relaxation. c) Receptor dependent arterial contraction. d) Receptor independent arterial contraction.

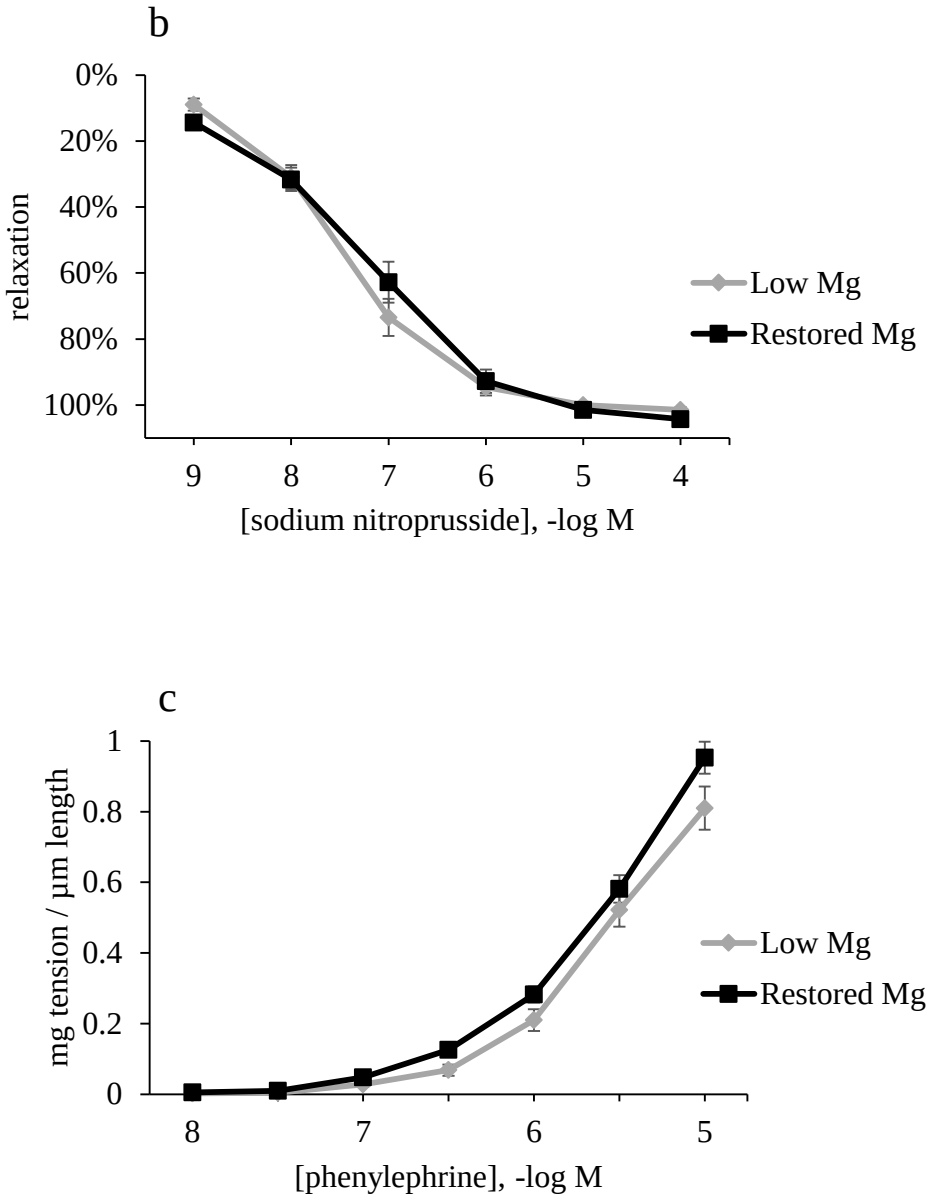

Figure 8 continued

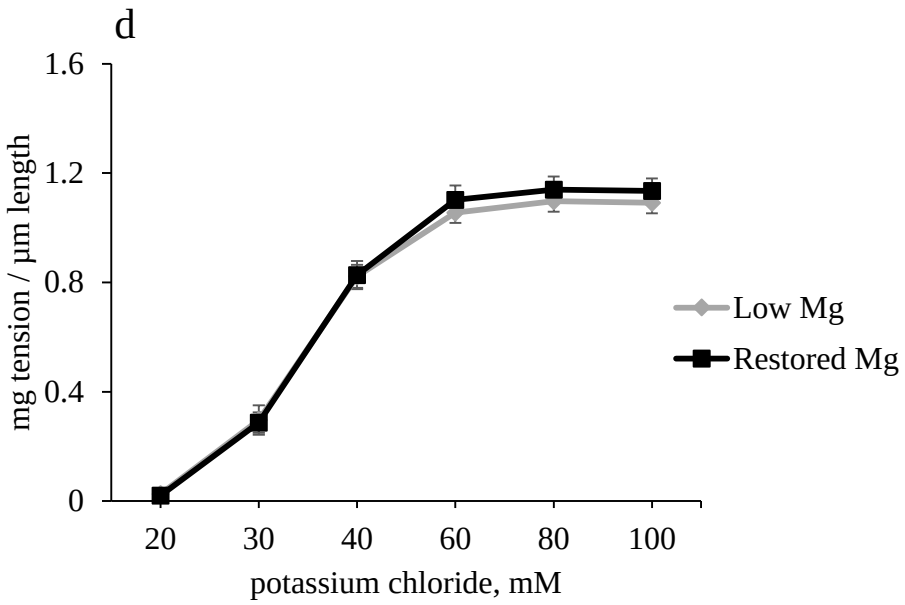

Figure 8 continued

DISCUSSION

Summary of main findings

Mg Citrate and Mg Bisglycinate restored bone and heart Mg tissue levels in a mouse model better than Mg Malate. After 3 weeks on low Mg diets, mice had significantly greater body fat and reduced energy expenditure compared to mice that were switched to restored Mg diets. These differences can likely be attributed to increased activity levels in the restored Mg group vs. the low Mg group. In liver tissue, reduced glutathione, inosine, and glycine were higher in the restored Mg group, while 22:0 fatty acid was higher in the low Mg group.

Mg absorption

Brilla, Frederickson, and Lombardi (1989) reported that the skeletal muscle Mg concentration is protected at the expense of bone Mg and other slowly exchanging pools of magnesium. The results from the muscle magnesium concentration test support this finding as the muscle Mg was maintained at the same level between the 100 mg/kg Mg Oxide groups and the 500 mg/kg Mg Bisglycinate group, while the bone Mg was lower in the 100 mg/kg Mg Oxide group than in the 500 mg/kg Mg Citrate group. A limitation of our analysis, however, is that due to limitations on tissue amounts, gastrocnemius, soleus, and quadracep were pooled together for analysis. It is possible that each tissue may have different Mg profiles, thus leading to variability in these results.

Wallach (1988) reported that during hypomagnesemia, the heart, kidney, and

brain may lose small but significant amounts of magnesium while the liver appears not to lose magnesium. The results of the present study support these assertions as the liver Mg concentration was the same across all groups, while in the heart, the 100 mg/kg Mg Oxide group had significantly less Mg than the 500 mg/kg Mg Citrate or 500 mg/kg Mg Bisglycinate groups. The 500 mg/kg Mg Malate group had the lowest amount of heart and bone Mg and appears to deplete or fail to restore Mg. It has been known for over 30 years that those who die of heart disease have lower heart Mg than those who die from other causes (Elwood & Beasley, 1981). These results underscore the need for a continual optimum intake of Mg to maintain critical tissue levels.

Body composition

The higher fat mass and fat percentage seen in the low Mg group was likely the result of reduced activity. To our knowledge, this is the first study to report changes in energy expenditure and body composition in mice with low Mg diets. We saw higher activity in the restored Mg group during CLAMS testing as well as a higher energy expenditure and a higher rate of respiration. Few studies have assessed voluntary activity; however, Keen, Lowney, Gershwin, Hurely, and Stern (1987) reported that Mg depleted rats had reduced exercise endurance capacity

Metabolic chamber and metabolomics

Food intake was the same between the low Mg group and the restored Mg group, indicating the only difference in nutrient intakes was the Mg intake. The lack of difference in the glucose tolerance test between low Mg and restored Mg treatments demonstrates that magnesium restriction did not impair glucose tolerance, suggesting

normal cellular glucose uptake. This is also in contrast to previous studies that suggest low Mg status is correlated with insulin resistance and diabetes (Rosanoff et al., 2012). Perhaps more severe Mg depletion is required to impair glucose tolerance. Finally, the glucose tolerance in this study was in line with the measured Respiratory Exchange Ratio (RER) that indicated similar macronutrient utilization between groups. Taken together, these data suggest that carbohydrate uptake and utilization is not altered by this dietary approach.

The greater levels of reduced glutathione in the restored Mg group vs. the low Mg group (metabolomics data) indicate an inhibition of the two Mg-dependent enzymes needed to synthesize glutathione, glutamyl cysteine synthetase, and glutathione synthetase. This finding supports similar results seen in an earlier study (Regan & Guo, 2001). Synthesis of glycine is not directly Mg dependent, though glycine is one of the three amino acids making up reduced glutathione. The reduction in glutathione could be in part due to the lower concentrations of glycine seen. Lack of significance for the majority of the metabolites in the metabolomics results is likely influenced by the lack of difference in liver tissue magnesium seen in the tissue analysis results.

Vascular function

In spite of following a low dietary Mg protocol that reduced heart Mg concentations and has been previously reported to compromise Mg status in mice (Feillet-Coudray et al., 2004), we found no changes in arterial function. A potential confounder of the vessel reactivity experiments was the presence of Mg in the buffers used to keep the vessels functional during the experiments. Plasma Mg levels in rats have been reduced to .42 mmol by following a diet with the same low Mg concentration for a

similar period of time as in the present study (Keen et al., 1987). Mg is known to enter the vascular smooth muscle cells when present extracellularly and, when present at levels of 4.8 mmol, can more than double the intracellular concentration of Mg in only 5 minutes (Zhang, Chang, Altura, & Altura, 1992). In the present study, vessels were incubated in buffer containing 1 mmol Mg for 3-6 hours during the vessel experiments. Altura and Altura (1990) indicated that extracellular Mg can effect the tone and contractility of the vascular smooth muscle cells by altering the intracellular binding and transport of calcium.

Conclusion

The results of the present study show a need for continuous optimal intake of Mg in order to maintain tissue levels of magnesium, and we found that in general, tissue Mg levels are protected in liver and muscle. Mg Citrate and Mg Bisglycinate restored bone and heart Mg levels better than Mg Malate, suggesting greater bioavailability of these isoforms. The lower activity level seen in the low Mg group relative to the restored Mg group was a striking finding, and appears to explain the increased body fat in these mice. However, it is unknown why Mg depletion reduces voluntary physical activity; therefore, this phenomenon warrants further exploration as to the mechanistic cause. Finally, the impact that dietary Mg had on body fat in this study may have clinical relevance when one considers the current epidemic of obesity in the United States. Future studies should address this relationship.

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